RESPONSE

To Mr. Seiji Sakano, Examiner of the Patent Office

1 Indication of International Application
PCT/JP2005/004274

2 Applicant

Name Kyoto University

Address 36-1, Yoshida-honmachi, Sakyo-ku, Kyoto-shi, Kyoto 606-8501 Japan

Nationaligy Japan

Domicile Japan

3 Agent

Name TAKASHIMA Hajime

Address Meiji Yasuda Seimei Osaka Midosuji Bldg., 1-1, Fushimimachi 4-chome, Chuo-ku, Osaka-shi, Osaka 541-0044 Japan

Agent Registration No. 100080791

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5 Contents of response

(1) In the Written Opinion dated June 14, 2005 (mailing date), the Examiner cites the following documents 1 and 2 and holds that claims 10 and 11 and claims 19, 21 and 33 lack an inventive step

Document 1: T. Nakamura, et. al , DANCE, a novel secreted RGD protein expressed in developing, atherosclerotic, and balloon-injured arteries, J Biol Chem, 274(32), 1999, 22476-83

Document 2: X. Liu, et al, Elastic fiber homeostasis requires lysyl oxidase-like 1 protein, Nat Genet, 36(2), 2004.02, 178-82

The Examiner also holds that the claims are not clearly described, and that the inventions described in the claims are neither fully supported in the Description nor disclosed clearly and sufficiently to the extent that those of ordinary skill in the art can practice the invention.

The Applicant cannot agree with such view of the Examiner and would respectfully present the opinion in the

following.

(2) The present invention

i) Amendments

The claims have been amended as shown in the Amendment of the even date.

The amendment to claim 1 is supported by the Description, page 11, lines 16-23, page 12, lines 6-30 and the like.

The amendment to claim 2 is supported by the Description, page 12, line 31 to page 13, line 2, and the like.

The amendment to claim 4 is supported by the Description, page 14, line31 to page 15, line 3, and the like.

The amendment to claim 5 is supported by the Description, page 11, lines 16-23, page 12, lines 6-17 and page 13, lines 3-13, and the like.

The amendment to claim 6 is supported by the Description, page 13, lines 23-28, and the like.

The amendment to claim 8 is supported by the Description, page 15, lines 4-10, and the like.

The addition of claims 35-37 is supported by the Description, page 45, line 23 to page 46, line 3, page 46, lines 15-20, and the like.

Other amendments are of formal nature and supported by the specification and the like as originally filed.

As shown above, the amendments made by the Amendment of even date do not introduce a new matter.

ii) The gist of the present invention

As stated in the Scope of Claims, the invention of this application relates to a polypeptide obtained by cleaving DANCE (also referred to as fibulin-5) with a DANCE-specific protease, a polynucleotide that encodes the polypeptide, a method of cleaving DANCE, an antibody having specific affinity for the polypeptide, a method of measuring the amount of DANCE cleaved, an assay reagent for the amount of DANCE cleaved, a DANCE mutant and a polynucleotide having a nucleotide sequence

that encodes the same, DANCE complexes (DANCE homo-complex and DANCE complex comprising DANCE and LTBP2) and methods of preparing the same, screening methods (a screening method for a substance capable of regulating the activity of a DANCE-specific protease, a screening method for a substance capable of regulating the formation of a DANCE complex, and a screening method for a DANCE-specific protease), a kit for forming a DANCE complex, a method of identifying DANCE-specific protease expression cells, a fraction having DANCE cleavage activity, and the like.

The points to note in the invention of this application reside in 1) the finding that DANCE (full-length DANCE) is cleaved, resulting in a particular cleaved form of DANCE (hereinafter the carboxy-terminal side polypeptide resulting from the cleavage of DANCE is abbreviated as "the C-terminal fragment" and the amino-terminal side polypeptide as "the N-terminal fragment" as required), 2) the finding that a DANCE-specific protease having DANCE cleavage activity exists, 3) the demonstration of the relationship between the cleavage of DANCE and the regulation of the formation of elastic fibers, 4) the finding that the regulation of the formation of elastic fibers by cleavage of DANCE can result from differences in the capability of forming a higher complex between full-length DANCE and cleaved forms of DANCE, and the like. These findings enable the development of a pharmaceutical of a new mechanism of action allowing the regulation of the formation of elastic fibers, determinations of the status of the formation of elastic fibers, and the like. Therefore, it is evident that the invention of this application is outstandingly excellent.

(3) Comparison of the invention of this application and prior art documents

[1] Claims 10 and 11

You have found the following:

"In Document 1, an antibody against DANCE is described (see Protein Expression and Antibodies, left column, page 22477). The antibody is undistinguishable from an antibody having specific affinity for a polypeptide cleaved with a DANCE-specific protease. Therefore, the inventions relating to these

claims do not involve in novelty or an inventive step in view of Document 1."

As the Examiner have found, an antibody against DANCE is described in Document 1. However, the antibody described in Document 1 is a rabbit anti-mouse/rat DANCE polyclonal antibody raised against the polypeptide CMTRPIKGPRDIQLDLEMITVN, which corresponds to the mouse and rat DANCE amino acids 406-426 (see lines 6-3 from bottom, Protein Expression and Antibodies, left column, page 22477). Note that the above-described polypeptide used to raise this polyclonal antibody is a partial peptide in the C-terminal fragment of DANCE.

Here, the antibody described in Claim 10 and the antibody described in Document 1 differ from each other in that the epitope for the antibody described in Claim 10 is the N-terminal fragment obtained by cleaving DANCE with a protease (i.e., a polypeptide comprising the amino acid sequence shown by SEQ ID NO:6 or substantially the same amino acid sequence thereas) or a partial peptide thereof, whereas the epitope for the antibody described in Document 1 is a partial peptide in the C-terminal fragment obtained by the cleavage (i.e., CMTRPIKGPRDIQLDLEMITVN). Therefore, the antibody described in Claim 10 is distinguishable from the antibody described in Document 1, and hence involves in novelty to Document 1.

Additionally, the antibody described in Claim 11 and the antibody described in Document 1 differ from each other in that the kind of antibody for the antibody described in Claim 11 is a monoclonal antibody, whereas the kind of antibody for the antibody described in Document 1 is a polyclonal antibody. Therefore, the antibody described in Claim 11 is distinguishable from the antibody described in Document 1, and hence involves in novelty to Document 1.

Furthermore, the antibodies described in Claims 10 and 11, particularly the antibody described in Claim 10, is not obvious to those skilled in the art. This is because motivation for preparing an antibody against the N-terminal fragment of DANCE cannot arise without the finding of the invention of this application that DANCE is cleaved with a protease, but if this finding is unknown, there is no

motivation for preparing such an antibody. In particular, because the antibody described in Document 1 exhibits good reactivity, as shown in FIG. 2 thereof (in other words, the partial peptide used is of good quality for an epitope), it is considered that those skilled in the art will of course use this partial peptide in the C-terminal fragment when preparing an antibody against DANCE.

Additionally, the antibodies described in Claims 10 and 11 relate to antibodies that specifically recognize cleavage ends but do not recognize full-length DANCE. Such an antibody is an antibody specific for a cleaved form of DANCE, and is highly useful for quantifying the amount of DANCE cleaved.

Hence, we believe that the inventions relating to Claims 10 and 11 involve in novelty and an inventive step to Document 1.

[2] Claims 19, 21, and 33

You have found the following:
"In Document 2, it is stated that LOXL1 (lysyl oxidase-like 1) binds to fibulin-5 (DANCE) (see Figure 5). From the description of Document 2, those skilled in the art can easily arrive at the fact that DANCE forms a complex with lysyl oxidase. Therefore, the inventions relating to these claims do not involve in an inventive step in view of Document 2."

In response to the Examiner's judgment, we have removed 'lysyl oxidase' and 'polynucleotide having a nucleotide sequence that encodes lysyl oxidase' from Claims 19, 21, and 33.

Hence, we believe that the inventions relating to Claims 19, 21, and 33 involve in an inventive step to Document 2.

[3] Others (Claims 2, 4, 6, 8, and 35-37)

Regarding the inventions of polypeptides relating to mouse and rat cleaved forms of DANCE and polynucleotides that encode the polypeptides, added to Claims 2, 4, 6, and 8 in the amendment by the written amendment dated the same day, there is no statement or suggestion in Documents 1 and 2.

Regarding the inventions relating to Claims 35-37, added by the amendment by the written amendment dated the same day,

i.e., the inventions relating to fractions having DANCE cleavage activity, there is no statement or suggestion in Documents 1 and 2.

Hence, we believe that the inventions relating to Claims 2, 4, 6, 8, and 35-37 involve in novelty and an inventive step to Documents 1 and 2.

(4) Clarity of the Scope of Claims, Description, and Drawings, or sufficient evidence for supporting the Scope of Claims by the Description

[1] Claims 1-15, 22-24, and 28-34

You have found that the intended uses of the series of inventions relating to Claims 1-15, 22-24, and 28-34 remain unclear, and therefore the inventions cannot be said to be fully supported in the Description, or to be disclosed clearly and sufficiently to the extent enabling an embodiment thereof by an expert in the relevant technical field. We think that the Examiner's conclusion above is based on the Examiner's thought that the correlation between the cleavage of DANCE by a protease and the regulation of the formation of elastic fibers in a living organism is not evident, judging from the description in the Expert Opinion. However, the correlation between the cleavage of DANCE by a protease and the regulation of the formation of elastic fibers in a living organism is fully supported by the statements in Examples and elsewhere in the Description and common general technical knowledge in the relevant technical field. In Terms (a) to (d) below, the reasons for our judgment are described in detail.

(a) Relationship between the formation of a cleaved form of DANCE from full-length DANCE and a DANCE-specific protease in a living organism

The present inventors found that DANCE is cleaved with a DANCE-specific protease in vitro and in vivo, and that DANCE occurs in a living organism in two forms, i.e., full-length DANCE and a cleaved form of DANCE (C-terminal fragment) (see, for example, Examples 1 and 9). Thus, it is considered that if the activity of a DANCE-specific protease in a living organism is high, the cleavage of DANCE by the protease is promoted,

which in turn can result in a decrease in full-length DANCE and an increase in the cleaved form of DANCE in the living organism. On the other hand, it is considered that if the activity of a DANCE-specific protease in a living organism is low, the cleavage of DANCE by the protease is suppressed, which in turn can result in an increase in full-length DANCE and a decrease in the cleaved form of DANCE in the living organism.

Therefore, we think that the Examiner will understand the relationship between the formation of a cleaved form of DANCE from full-length DANCE and a DANCE-specific protease in a living organism from the results of research by the present inventors.

(b) Full-length DANCE can have the capability of forming elastic fibers in a living organism

From findings in mice and humans obtained before the filing of this application, it had been known that DANCE can mediate the formation of elastic fibers in a living organism (i.e., in vivo). For example, it had been reported that DANCE (also referred to as Fibulin-5; this statement is omitted below) knockout mice exhibit an abnormality in the formation of elastic fibers (see, for example, lines 14-29, page 2 of the Description). It had also been reported that a missense mutation of DANCE and tandem duplication of a particular region thereof in humans can be causes of abnormalities in the formation of elastic fibers, such as cutis laxa (see, for example, the Abstracts of Reference Documents 1 and 2). However, it had remained unknown whether or not the formation of elastic fibers can be reversibly regulated by DANCE even after ontogeny. This was because of the possibility that the above-described failures of the formation of elastic fibers in mice and humans may result from the deficiency of DANCE at a particular stage of the developmental process, and may be irreversible or cannot be ameliorated even when DANCE in the living organism is increased after ontogeny.

Against this background, the present inventors found that full-length DANCE has the capability of forming elastic fibers in vitro (see, for example, Reference Example 2). This

strongly suggests that the formation of elastic fibers can be reversibly promoted by increasing full-length DANCE in a living organism even after ontogeny (i.e., that elastic fibers can be regenerated).

Therefore, we think that the Examiner will understand, from the results of research by the present inventors, that full-length DANCE is considered to be able to have the capability of forming elastic fibers in a living organism.

(c) Cleaved forms of DANCE cannot have the capability of forming elastic fibers in a living organism

(c1) The C-terminal fragment of DANCE cannot have the capability of forming elastic fibers in a living organism

The present inventors found that the C-terminal fragment of DANCE (identical to ΔND DANCE disclosed in Examples of the specification) is substantially free from the activity to form elastic fibers (see, for example, Reference Examples 1 and 2). Therefore, it is considered that the C-terminal fragment resulting from the action of a DANCE-specific protease cannot have the capability of forming elastic fibers in a living organism.

(c2) The N-terminal fragment of DANCE cannot have the capability of forming elastic fibers in a living organism

The above-described finding that the C-terminal fragment of DANCE cannot have the capability of forming elastic fibers suggests that a particular region contained in the N-terminal fragment can be necessary for the formation of elastic fibers. Therefore, when taking into consideration this finding alone, it is considered that not only full-length DANCE, but also the N-terminal fragment of DANCE, can have the capability of forming elastic fibers. However, because it is considered that a particular region in the C-terminal fragment is also necessary for the formation of elastic fibers, the N-terminal fragment per se is considered to be unable to have the capability of forming elastic fibers. Hereinafter, the reasons are described in detail.

The first reason is that because the N-terminal fragment of DANCE lacks as many as five units of calcium-binding EGF

(cbEGF)-like motif (present in the central domain of DANCE, and contained in the C-terminal fragment), which is conserved among the members of the family of fibulin, an extracellular matrix protein, and which is hence considered to be functionally important (see, for example, FIG. 1D of Cited Document 1, and lines 13-19, page 13 and FIG. 4 of the Description), this type of DANCE (i.e., N-terminal fragment) is quite unlikely to have the capability of forming elastic fibers, which is seemingly the major function thereof.

The second reason is that because findings in humans obtained before the filing of this application suggest that a particular region in the C-terminal fragment may play an important role in the formation of elastic fibers, the N-terminal fragment is unlikely to be able to have the capability of forming elastic fibers. For example, it has been reported that the missense mutation S227P of DANCE in humans [i.e., a mutation wherein the amino acid at the 227 position of DANCE (present in the C-terminal fragment), i.e., serine, has been substituted by proline] can be a cause of abnormalities in the formation of elastic fibers, such as cutis laxa (see, for example, the Abstract of Reference Document 1). It has also been reported that duplication of the 5-8 exons can be a cause of abnormalities in the formation of elastic fibers, such as cutis laxa (see, for example, the Abstract of Reference Document 2). Hence, since a mutation in a particular region in the C-terminal fragment causes abnormalities in the formation of elastic fibers, it is considered that a particular region (normal particular region) in the C-terminal fragment is necessary for the formation of elastic fibers. Therefore, it is considered that the N-terminal fragment, which does not comprise such a particular region, cannot have the capability of forming elastic fibers.

The third reason is that because the C-terminal fragment of DANCE comprises both a region for binding to an enzyme of the lysyl oxidase family and a region for binding to the microfibril constituent LTBP2, which regions can play an important role in the formation of elastic fibers, it is considered that the N-terminal fragment, which does not comprise such binding regions, cannot have the capability of

forming elastic fibers. During the formation of elastic fibers, it is considered to be important that elastin deposits along the fibers called microfibril and is crosslinked by an enzyme of the lysyl oxidase family (LOX, LOXL1-4) (see, for example, lines 28-33, page 1 of the Description). However, no factors have been known to date that can mediate the deposition of elastin to microfibril. However, the present inventors found that DANCE, which binds to both an enzyme of the lysyl oxidase family and the microfibril constituent LTBP2, can be such a mediator, as described in (4) [8] (a) below. By the way, the region for binding to an enzyme of the lysyl oxidase family and the region for binding to the microfibril constituent LTBP2 are contained in the C-terminal fragment (see, for example, Example 6 and lines 16-19, page 60 of the Description). Therefore, the N-terminal fragment of DANCE, which does not comprise these binding regions capable of playing an important role in the deposition of elastin to microfibril, are quite unlikely to have the capability of forming elastic fibers.

The fourth reason is that the N-terminal fragment is very likely to be decomposed. A Western blotting using an antibody that recognizes a sequence in the N-terminal fragment detects full-length DANCE but does not detect the N-terminal fragment. The same applies not only to in vitro DANCE overexpression experiments, but also to tissue samples from a living organism. Therefore, it is considered that the N-terminal fragment, which is thus very likely to be decomposed, cannot have the capability of forming elastic fibers.

We think that the Examiner will understand that it is considered, from the results of research by the present inventors and findings already known before the filing of this application, that cleaved forms of DANCE (N-terminal fragment and C-terminal fragment) cannot have the capability of forming elastic fibers in a living organism, as described in detail above.

(d) On the findings derived from (a) to (c) above

Because it is stated that (b) full-length DANCE can have the capability of forming elastic fibers in a living organism,

and that (c) cleaved forms of DANCE (N-terminal fragment and C-terminal fragment) cannot have the capability of forming elastic fibers in a living organism, the correlation between the cleavage of DANCE and the regulation of the formation of elastic fibers is evident; furthermore, because it is also stated that (a) there is a relationship between the formation of a cleaved form of DANCE from full-length DANCE and a DANCE-specific protease in a living organism, we think that the correlation between the cleavage of DANCE by the protease and the regulation of the formation of elastic fibers in a living organism is fully demonstrated. Hence, it can be said that the regulation of the activity of a DANCE-specific protease is associated with the regulation of the formation of elastic fibers, and that a substance capable of regulating the activity of a DANCE-specific protease is a regulator of the formation of elastic fibers, and we think that the intended use of the screening method for the substance is evident (Claim 22). We also think that the utility of the polypeptide obtained by cleaving DANCE, antibody against the polypeptide, DANCE mutants and the like used in the screening method is evident.

In addition, for caution's sake, a polypeptide obtained by cleaving DANCE is useful as, for example, an index for screening, or as an inhibitor of the formation of elastic fibers (considered to be capable of having a dominant negative effect). A polynucleotide that encodes the polypeptide is useful in, for example, efficiently and conveniently producing a polypeptide having the above-described utility (enabling the production through one step for producing a cleaved form of DANCE in a transformant, rather than through two steps for producing full-length DANCE in a transformant, and cleaving the full-length DANCE produced). An antibody against the polypeptide is useful in, for example, monitoring of the cleavage of DANCE in screening. A DANCE mutant is useful as, for example, a form of full-length DANCE that cannot be cleaved with a DANCE-specific protease, and that has an extended half-life in a living organism.

Therefore, we think that the series of inventions relating to Claims 1-15, 22-24, and 28-34 are fully supported

in the Description and disclosed clearly and sufficiently to the extent enabling an embodiment thereof by an expert in the relevant technical field.

[2] Claims 1 and 5

In response to the Examiner's judgment, we have amended Claims 1 and 5 to specify the degree of sequence identity to not less than 90%, and also to specify the kind of the same quality of activity.

We think that Claims 1 and 5 as amended are clearly stated, and that the inventions relating to these claims are fully supported in the Description and disclosed clearly and sufficiently to the extent enabling an embodiment thereof by an expert in the relevant technical field.

[3] Claims 3 and 7

In response to the Examiner's judgment, we have amended the statement "having •• sequence" in Claims 3 and 7 to the statement "consisting •• sequence".

We think that Claims 3 and 7 as amended are clearly stated.

[4] Claim 12

In response to the Examiner's judgment, we have amended Claim 12 to specify the purpose of measuring the amount cleaved, and to make a limitation to cleavage with a DANCE-specific protease.

We think that Claim 12 as amended is clearly stated, and that the invention relating to this claim is fully supported in the Description and disclosed clearly and sufficiently to the extent enabling an embodiment thereof by an expert in the relevant technical field.

[5] Claim 24

In response to the Examiner's judgment, we have amended Claim 24 to exclude humans from the scope of the animals.

We think that Claim 24 as amended has resolved the problem pointed out by the Examiner.

[6] Claims 28 and 30-32

You have found that it remains unclear what compounds are "a regulator of the formation of elastic fibers" and "a DANCE-specific protease". However, as described in Example 3, we have shown that a DANCE-specific protease is a kind of serine protease, and an inhibitor thereof can be deemed "a regulator of the formation of elastic fibers". Those skilled in the art can easily realize from the disclosure in the Description and common general technical knowledge in the relevant technical field what compounds are "a regulator of the formation of elastic fibers" and "a DANCE-specific protease".

Hence, we think that Claims 28 and 30-32 are clearly stated, and that the inventions relating to these claims are fully supported in the Description and disclosed clearly and sufficiently to the extent enabling an embodiment thereof by an expert in the relevant technical field.

[7] Claim 33

In response to the Examiner's judgment, we have amended the statement to "an expression vector comprising the polynucleotide (i.e., a DANCE or LTBP2 expression vector)" to specify the intended use of the kit, and to further clarify the intended use of the polynucleotide. Such an expression vector is useful for preparing a DANCE complex.

Hence, we think that Claim 33 is clearly stated, and that the invention relating to this claim is fully supported in the Description and disclosed clearly and sufficiently to the extent enabling an embodiment thereof by an expert in the relevant technical field.

[8] Claims 16-21, 25-28, and 33

You have found that it remains unclear what are the intended uses of the series of inventions relating to Claims 16-21, 25-28, and 33. We think that the Examiner's conclusion is based on the Examiner's belief that the correlation between a DANCE complex and the regulation of the formation of elastic fibers is not evident, judging from the statements of the Expert Opinions. However, judging from the statements in

Examples and elsewhere in the Description and common general technical knowledge in the relevant technical field, the correlation between a DANCE complex and the regulation of the formation of elastic fibers is sufficiently described. Hereinafter, the reasons are described in detail in (a) and (b) below.

(a) Relationship between a complex comprising DANCE and LTBP2 and the formation of elastic fibers

During the formation of elastic fibers, it is considered to be important that elastin deposits along the fibers called microfibril and is crosslinked by an enzyme of the lysyl oxidase family (LOX and LOXL1-4) (see, for example, lines 28-33, page 1 of the Description). Although no factor has been known to date that can mediate the deposition of elastin to microfibril, the present inventors found that DANCE, which binds to both the microfibril constituent LTBP2 and an enzyme of the lysyl oxidase family, can be such a mediator. By first explaining the relationship between microfibril and DANCE, and subsequently explaining the relationship between enzymes of the lysyl oxidase family and DANCE, the reasons why DANCE is considered to be a factor capable of mediating the deposition of elastin to microfibril are stated below.

• Relationship between microfibril and DANCE

The present inventors found that DANCE binds to the microfibril constituent LTBP2 via the central domain thereof (present in the C-terminal fragment) (see, for example, Examples 5 and 6 and FIGS. 9 and 10). Although a large number of factors, including fibrillin 1 and fibrillin 2, are known as microfibril constituents, it is unlikely that fibrillin 1 and fibrillin 2 are involved in the formation of elastic fibers, with no reports available on a microfibril constituent capable of being involved in the formation of elastic fibers (see, for example, line 33, page 1 - line 13, page 2, and line 25, page 60 to line 1, page 61 of the Description). The present inventors also confirmed that DANCE does not bind to microfibril constituents other than LTBP2, such as fibrillin 1 and fibrillin 2 (see, for example, Example 5 and FIG. 9). These findings suggest that DANCE may specifically interact

with LTBP2, out of the microfibril constituents, during the formation of elastic fibers.

• Relationship between enzymes of the lysyl oxidase family and DANCE

Because of the fact that LOXL1 knockout mice, like DANCE knockout mice, suffer elastic fiber dysplasia, that in DANCE knockout mice, LOXL1 is no longer localized on elastic fibers, and that a region in the C-terminal fragment of DANCE is necessary for the binding to LOXL1, it has been reported that DANCE is capable of functioning as an adapter to anchor LOXL1 at a due position (see, for example, line 30, page 2 to line 3, and line 18-19, page 60, of the Description). The present inventors also found that the phenotype of lysyl oxidase (LOX) knockout mice is highly similar to the phenotype of DANCE knockout mice, and that DANCE binds to LOX (see Example 7).

• DANCE is capable of mediating the deposition of elastin to microfibril

DANCE is capable of specifically interacting with the microfibril constituent LTBP2 in the formation of elastic fibers, as described above. DANCE is also capable of functioning as an adapter to anchor an elastin-crosslinking enzyme at a due position in the formation of elastic fibers, as described above. Therefore, the results of research by the present inventors and findings already known before the filing of the present application suggest that LTBP2 may localize the elastin-crosslinking enzyme on microfibril by anchoring DANCE on microfibril, thus helping the deposition and crosslinking of elastin along microfibril (see, for example, Discussion 1). That is, it is considered that as DANCE binds to the microfibril constituent LTBP2 and an enzyme of the lysyl oxidase family, a higher complex of microfibril and elastin (a higher complex comprising at least microfibril constituent LTBP2/DANCE/lysyl oxidase family enzyme/elastin) via DANCE as the mediator is formed, which can mediate the deposition of elastin to microfibril.

Therefore, we think that the correlation of a complex comprising DANCE and LTBP2 and the regulation of the formation

of elastic fibers is evident from the statements in Examples and elsewhere in the Description and common general technical knowledge in the relevant technical field.

(b) Relationship between a DANCE homo-complex and the formation of elastic fibers

The present inventors identified DANCE as the major DANCE-binding protein present in smooth muscle culture supernatant, and found that units of DANCE bind to each other to form a homo-complex (dimer or multimer), and that this binding of units of DANCE to each other is via an N-terminal domain (i.e., a region in the N-terminal fragment) (see, for example, Examples 5 and 6).

By the way, because full-length DANCE can have the capability of forming elastic fibers in a living organism, and also because the C-terminal fragment (i.e., ΔND-DANCE) cannot have the capability of forming elastic fibers in a living organism (see, for example, Reference Example 2), it is considered that at least one particular region considered to be important to the formation of elastic fibers is contained in the N-terminal fragment.

As the functional region contained in the N-terminal fragment, the integrin-binding site (RGD motif) and the homo-complex formation site found by the present inventors at the present occasion can be mentioned. However, because the integrin-binding site has cell adhesion promoting activity, it is considered to mediate the binding of elastic fibers and cells, but not to have the capability of forming elastic fibers. Note that the present inventors actually confirmed that when a full-length DANCE mutant confirmed to hardly bind to integrin (integrin-binding site RGD mutated to RGE) is cultured in a serum-free medium containing human skin fibroblasts, elastic fibers are formed as with full-length DANCE (non-mutant). Therefore, because it is considered that the homo-complex formation site can correspond to at least one particular region considered to be important to the formation of elastic fibers, a DANCE homo-complex is considered to be capable of playing an important role in the regulation of the formation of elastic fibers.

Elastic fibers are extracellular fibers responsible for tissue elasticity in elastic tissues such as the lungs, arteries, and skins. The elasticity of elastic fibers is due to crosslinked elastin protein; elastic fibers cannot exhibit the function thereof unless units of elastin are crosslinked in a uniform streak on microfibril. The fact that elastin protein cannot assume the normal structure without DANCE has been demonstrated in DANCE gene-deficient mice (see, for example, line 6, left column, page 173, - line 2, left column, page 174, of Cited Document 1). The molecular mechanism of the construction of elastic fibers by DANCE can be explained by the DANCE complexes described in the present invention, wherein DANCE binds to LTBP2, which is a microfibril constituent protein, and to lysyl oxidase enzyme, which is an elastin-crosslinking enzyme, to form a complex, and hence to uniformly crosslink units of elastin along microfibril.

To facilitate the Examiner's understanding of the matters concerning (a) and (b) above, we are submitting Document 3 describing a DANCE action model.

Therefore, we think that the correlation between a DANCE homo-complex and the regulation of the formation of elastic fibers is evident from the statements in Examples and elsewhere in the Description and common general technical knowledge in the relevant technical field.

We think that from (a) and (b) above, the correlation between a DANCE complex and the regulation of the formation of elastic fibers is evident. Hence, it can be said that the regulation of the formation of a DANCE complex is associated with the regulation of the formation of elastic fibers, and that a substance capable of regulating the formation of a DANCE complex is a regulator of the formation of elastic fibers, and we think that the intended use of the screening method for the substance (Claim 25) is evident, and that the utility of the DANCE complexes and the like used in the screening method is clear.

Therefore, we think that the inventions relating to Claims 16-21, 25-28, and 33 are fully supported in the Description and disclosed clearly and sufficiently to the extent enabling an embodiment thereof by an expert in the

relevant technical field.

[9] Others (Claims 2, 4, 6, 8, and 35-37)

The inventions of polypeptides relating to mouse and rat cleaved forms of DANCE and polynucleotides that encode the polypeptides, added to Claims 2, 4, 6, and 8 in the amendment by the written amendment dated the same day, are sufficiently supported in the Description, and are disclosed clearly and sufficiently to the extent enabling an embodiment thereof by an expert in the relevant technical field. Human, mouse, and rat DANCEs exhibit extremely high homology among these species (see, for example, Fig. 1C of Cited Document 1). In particular, the amino acids in the vicinity of the cleavage site by a DANCE-specific protease (cleaved between the 77th Arg and the 78th Gly) are 100% conserved (see, for example, Fig. 1C of Cited Document 1). Therefore, because a substrate portion recognized by a DANCE-specific protease is highly conserved among human, mouse and rat DANCEs, it can be considered that mouse and rat DANCEs are also cleaved at the same position. Note that the present inventors have confirmed that cleaved forms of DANCE are present in mice as well (see, for example, Example 1).

The inventions relating to Claims 35-37, added by the amendment by the written amendment dated the same day, i.e., the inventions relating to fractions having DANCE cleavage activity, are fully supported in the Description and disclosed clearly and sufficiently to the extent enabling an embodiment thereof by an expert in the relevant technical field. For example, those skilled in the art can easily embody the inventions relating to Claims 35-37 by referring to a method of fractionation with DANCE cleavage activity as the index (see, for example, line 23, page 45 to line 3, page 46 of the Description), a method of cleaving DANCE (see, for example, line 7, page 21 to line 29, page 23 of the Description), methods of measuring the amount of DANCE cleaved and cleavage activity (for example, line 12, page 26 to line 3, page 29 of the Description), and common general technical knowledge in the relevant technical field.

Hence, we think that Claims 2, 4, 6, 8, and 35-37 are

clearly stated, and that the inventions relating to these claims are fully supported in the Description and disclosed clearly and sufficiently to the extent enabling an embodiment thereof by an expert in the relevant technical field.

(5) Conclusion

As described in detail in the above, we believe it is appreciated that the present invention has novelty and inventive step over the cited references 1 and 2 and that the claims, Description and drawings are definite, and the claims are fully supported by the Description.

6 List of annexed documents

(1) Reference 1: Loeys, B. et al., Human Molecular Genetics, 2002, Vol. 11, No. 18 2113-2118 1 copy

(2) Reference 2: Markova, D. et al., Am. J. Hum. Genet. 72: 998-1004, 2003 1 copy

(3) Reference 3: DANCE working model diagram